

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A method of identifying ~~an environmental stimulus or a gene that alters the lifespan of an organism, said method consisting essentially of:~~

providing a control cell culture and one or more test cultures, wherein the one or more test cell cultures but not the control cell culture comprise either ~~(i) mother yeast cells that possess a genotype modification of either a non-essential gene or an essential gene, in which case the genotype modification is non-lethal; (ii) mother yeast cells that are exposed to an environmental stimulus other than a pro-oxidant; or (iii) mother yeast cells that possess a genotype modification of either a non-essential gene or an essential gene, in which case the genotype modification is non-lethal, and are exposed to an environmental stimulus other than a pro-oxidant;~~

culturing the control cell cultures and one or more test cell cultures ~~under conditions on a growth medium that allows~~ whereby mother yeast cells ~~can to replicate but not and daughter yeast cells cannot;~~ and

determining whether the mother yeast cells in the one or more test cell cultures exhibit a change in replicative lifespan when compared to the mother yeast cells in the control cell culture, wherein an increase in the replicative lifespan for mother yeast cells of a test cell culture indicates that the genotype modification, ~~the environmental stimulus, or the combination thereof,~~ increases the replicative lifespan of an organism ~~exposed to the environmental stimulus,~~ possessing the genotype modification, ~~or the combination thereof and a decrease in the replicative lifespan for mother yeast cells of a test cell culture indicates that the genotype modification decreases the replicative lifespan of an organism possessing the genotype modification.~~

2. (Cancelled)

3. (Currently Amended) The method according to claim 1 ~~2~~ wherein the growth medium of the control cell culture and the one or more test cell cultures is free of galactose.

4. (Cancelled)

5. (Previously Presented) The method according to claim 59 wherein the promoter responsive to growth medium conditions is a promoter responsive to presence of galactose.

6. (Original) The method according to claim 5 wherein the promoter responsive to galactose presence is a *GAL1*, *GAL7*, or *GAL10* promoter.

7. (Previously Presented) The method according to claim 59 wherein the promoter operable only in mother cells is an HO endonuclease promoter.

8. (Previously Presented) The method according to claim 59 wherein a native gene encoding the protein required for replication is disrupted to prevent expression of the protein therefrom.

9. (Previously Presented) The method according to claim 59 wherein the protein required for replication is a cell cycle protein.

10. (Currently Amended) The method according to claim 9 wherein the cell cycle protein is ~~selected from the group of~~ *CDC2*, *CDC3*, *CDC4*, *CDC6*, *CDC7*, *CDC8*, *CDC9*, *CDC10*, *CDC13*, *CDC16*, *CDC20*, *CDC23*, *CDC24*, *CDC26*, *CDC27*, *CDC28*, *CDC34*, *CDC42*, and *CDC53*.

11. (Cancelled)

12. (Currently Amended) The method according to claim 1 ~~44~~ wherein the genotype modification is selected from the group of a deletion mutant, an overexpression mutant, an addition mutant, or encoding a mutant protein.

13-15. (Cancelled)

16. (Original) The method according to claim 1 wherein said determining comprises:

performing growth curve analyses on both the control cell culture and the one or more test cell cultures, and

assessing whether a difference exists between the growth curves of the control cell culture and the one or more test cell cultures.

17. (Previously Presented) The method according to claim 16 wherein said culturing is carried out in a liquid growth medium.

18. (Original) The method according to claim 17 wherein said performing growth curve analyses is carried out by measuring optical density of the liquid growth medium containing the cells.

19. (Previously Presented) The method according to claim 1 wherein said determining comprises:

assessing colony size of colonies present in the control cell culture and colonies present in the one or more test cell culture, wherein colony size is proportional to the replicative lifespan of the mother cell.

20. (Previously Presented) The method according to claim 19 wherein said culturing is carried out on a solid growth medium.

21. (Original) The method according to claim 20 wherein said assessing is done manually.

22. (Original) The method according to claim 20 wherein said assessing is carried out by analyzing optical images.

23. (Currently Amended) The method according to claim 22 wherein said analyzing optical images comprises:

capturing an image of colonies present in the control cell culture and an image of each of the one or more test cell cultures; and

calculating the two-dimensional area ~~or a morphometric property~~ of colonies in each of the images, wherein the two-dimensional area ~~or the morphometric property~~ of a colony is proportional to the replicative lifespan of the mother cell.

24. (Currently Amended) The method according to claim ~~1~~ ¹⁴⁵ wherein the genotype modification is replacement of a yeast gene that regulates lifespan with a human homolog of the yeast gene.

25. (Withdrawn) The method according to claim 24 wherein the human homolog is *RAS*, *BAX*, *SIR2*, *WRN*, or *BS*.

26. (Previously Presented) The method according to claim 59 wherein the yeast strain is a homozygous diploid host strain of yeast carrying two identical copies of each of the two chimeric genes but having a mutation in one copy of the non-essential gene.

27. (Original) The method according to claim 1 wherein the one or more test cell cultures comprises greater than ten test cell cultures.

28. (Original) The method according to claim 1 wherein the one or more test cell cultures comprises greater than one-hundred test cell cultures.

29-58. (Cancelled)

59. (Currently Amended) A method of identifying ~~an environmental stimulus~~ ~~or a gene~~ that alters the lifespan of an organism, said method comprising:

providing a control cell culture and one or more test cultures each comprising mother and daughter cells possessing two chimeric genes encoding a protein required for replication, one gene under control of an inducible promoter responsive to growth medium conditions and the other gene under control of a promoter operable in mother cells but not daughter cells; wherein one or more test cell cultures but not the control cell culture comprise ~~either (i) mother yeast cells that possess a genotype modification of either a non-essential gene or an essential gene, in which case the genotype modification is non-lethal, (ii) mother yeast cells that are exposed to an environmental stimulus other than a pro-oxidant, or (iii) mother yeast cells that possess a genotype modification of either a non-essential gene or an essential gene, in which case the genotype modification is non-lethal, and are exposed to an environmental stimulus other than a pro-oxidant;~~

culturing the control cell cultures and one or more test cell cultures under conditions whereby mother yeast cells can replicate and daughter yeast cells cannot; and

determining whether the mother yeast cells in the one or more test cell cultures exhibit a change in replicative lifespan when compared to the mother yeast cells in the control cell culture, wherein an increase in the replicative lifespan for mother yeast cells of a test cell culture indicates that the genotype modification, ~~the environmental stimulus, or the combination thereof,~~ enhances the replicative lifespan of an organism ~~exposed to the environmental stimulus,~~ possessing the genotype modification, ~~or the combination thereof~~ and a decrease in the replicative lifespan for mother yeast cells of a test cell culture indicates that the genotype

modification decreases the replicative lifespan of an organism possessing the genotype modification.

60. (Previously Presented) The method according to claim 59 wherein said culturing is carried out in a growth medium that allows for mother cell replication but not daughter cell replication.

61. (Previously Presented) The method according to claim 60 wherein the growth medium of the control cell culture and the one or more test cell cultures is free of galactose.

62. (Previously Presented) The method according to claim 59 wherein the one or more test cell cultures comprise mother cells that possess a genotype modification involving a nonessential gene.

63. (Currently Amended) A The method according to claim 62 wherein the of identifying a gene that alters the lifespan of an organism, said method comprising:

providing a control cell culture and one or more test cultures each comprising mother and daughter cells possessing two chimeric genes encoding a protein required for replication, one gene under control of an inducible promoter responsive to growth medium conditions and the other gene under control of a promoter operable in mother cells but not daughter cells; wherein one or more test cell cultures but not the control cell culture comprise mother yeast cells that possess a genotype modification of a non-essential gene, which genotype modification is selected from the group of a deletion mutant, an overexpression mutant, an addition mutant, or encoding a mutant protein;

culturing the control cell cultures and one or more test cell cultures under conditions whereby mother yeast cells can replicate and daughter yeast cells cannot; and

determining whether the mother yeast cells in the one or more test cell cultures exhibit a change in replicative lifespan when compared to the mother yeast cells in the control cell culture, wherein an increase in the replicative lifespan for mother yeast cells of a test cell culture indicates that the genotype modification enhances the replicative lifespan of an organism possessing the genotype modification and a decrease in the replicative lifespan for mother yeast cells of a test cell culture indicates that the genotype modification decreases the replicative lifespan of an organism possessing the genotype modification.

64. (Previously Presented) The method according to claim 59 wherein said determining comprises:

performing growth curve analyses on both the control cell culture and the one or more test cell cultures, and

assessing whether a difference exists between the growth curves of the control cell culture and the one or more test cell cultures.

65. (Previously Presented) The method according to claim 64 wherein said culturing is carried out in a liquid growth medium.

66. (Previously Presented) The method according to claim 65 wherein said performing growth curve analyses is carried out by measuring optical density of the liquid growth medium containing the cells.

67. (Previously Presented) The method according to claim 59 wherein said determining comprises:

assessing colony size of colonies present in the control cell culture and colonies present in the one or more test cell culture, wherein colony size is proportional to the replicative lifespan of the mother cell.

68. (Previously Presented) The method according to claim 67 wherein said culturing is carried out on a solid growth medium.

69. (Previously Presented) The method according to claim 68 wherein said assessing is done manually.

70. (Previously Presented) The method according to claim 68 wherein said assessing is carried out by analyzing optical images.

71. (Currently Amended) The method according to claim 70 wherein said analyzing optical images comprises:

capturing an image of colonies present in the control cell culture and an image of each of the one or more test cell cultures; and

calculating the two-dimensional area ~~or a morphometric property of~~ colonies in each of the images, wherein the two-dimensional area ~~or the morphometric property of~~ a colony is proportional to the replicative lifespan of the mother cell.

72. (Previously Presented) The method according to claim 59 wherein the one or more test cell cultures comprises greater than ten test cell cultures.

73. (Previously Presented) The method according to claim 59 wherein the one or more test cell cultures comprises greater than one-hundred test cell cultures.

74. (Currently Amended) A method of identifying a gene that alters the lifespan of an organism, said method comprising:

providing a control cell culture and one or more test cultures, wherein the one or more test cell cultures but not the control cell culture comprise mother yeast cells that possess a genotype modification of either a non-essential gene or an essential gene, in which case the genotype modification is non-lethal;

culturing the control cell cultures and one or more test cell cultures ~~under conditions on a growth medium that allows whereby~~ mother yeast cells ~~can~~ to replicate ~~but not~~ and daughter yeast cells ~~cannot~~; and

determining whether the mother yeast cells in the one or more test cell cultures exhibit a change in replicative lifespan when compared to the mother yeast cells in the control cell culture, wherein an increase in the replicative lifespan for mother yeast cells of a test cell culture indicates that the genotype modification increases the replicative lifespan of an organism possessing the genotype modification and a decrease in the replicative lifespan for mother yeast cells of a test cell culture indicates that the genotype modification decreases the replicative lifespan of an organism possessing the genotype modification.